

REMARKS

Applicant wishes to thank the Examiner for courtesies extended during the recent telephonic interview. The interview concerned discussion of the final Office Action of August 25, 2004. In particular, the discussion concerned enablement of the claims with regard to autoimmune disease and cancer. The Examiner's suggestions have been incorporated into the current response. Applicant notes with appreciation the withdrawal of rejections made under 35 USC §112, second paragraph.

With this amendment, claims 1, 3, and 5 are the claims currently being examined. Claim 1 is the only claim currently being examined which is in independent form. Applicant incorporates by reference the Amendment of June 1, 2004. It is submitted that no new matter has been added to this application by way of this amendment.

Remarks Directed to Claim Objections

Claim 2 was objected to as being of improper dependent form for failing to further limit claim 1. Applicant hereby cancels claim 2 as directed by the Examiner. Applicant therefore requests withdrawal of the objection.

**Remarks Directed to Rejection of Claims 1, 3, and 5 under
35 U.S.C. §112, First Paragraph**

Claims 1, 3, and 5 are rejected under 35 U.S.C. §112, first paragraph as containing subject matter not described in the specification in such a way as to enable one skilled in the art to which it pertains or with which it is most nearly connected, to make and/or use the invention. Specifically, the claims have been rejected following consideration of several factors relevant to enablement as outlined in *In re Wands* (858 F.2d 731, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)).

Specific polymorphisms and transcription factor binding

The Examiner's assertion that the specification does not teach any specific haplotypes that contain polymorphisms that bind NF-IL6 transcription factor is repeated in the August 25, 2004 Office Action. Specifically, it is asserted that "[w]hile the specification teaches a luciferase assay with regard to a T or a C at position -844 and that the C allele showed almost twice the activity than the T allele, the specification does not teach how such is correlated to binding of NF-IL6 ..." (Office Action, January 28, 2004, p.5 and August 25, 2004, p.4)

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However, the specification does clearly teach specific haplotypes that contain polymorphisms that bind NF-IL6 transcription factor. For example, the specification teaches that “[t]he SNP at nt -844 is within the C/EBP β (NF-IL6) transcription factor and that different alleles of the C/EBP β element have different affinities for the transcription factor.” (p.35, lines 11-13) Further, in describing the EMSA analysis shown in Figure 2B the specification teaches that “[t]aken together, the putative C/EBP β region including -844 is indeed a C/EBP β element, different genotypes of FasL promoter have [the] dramatic[ally] different affinities for the C/EBP β .” (p.28, lines 12-14)

Applicant submits that the data discussed above, as well as data further detailed in the specification, teach specific sequences that contain polymorphisms that bind NF-IL6 transcription factor. Applicant therefore requests withdrawal of the rejection of the claims under 35 U.S.C. §112, first paragraph.

Metes and bounds of the promoter ‘region’

The Examiner has rejected the claims as not enabled since they are “drawn to haplotyping in a ‘region’ wherein the specification does not define the metes and bounds of said region.” However, the specification states that the “5’ promoter region sequence of the Fas ligand [is] known” and cites C. J. Holtz-Heppelmann et al., J. Biol. Chem. 1998, 273(8):4416-4423. (p. 40, lines 2-5) In addition, the specification includes the information that “[t]he human FasL gene consists of four exons and spans about 8 kb on chromosome 1q23” citing T. Takahashi et al., *International Immunology* 1994, 6(10):1567-74. (p.7, lines 2-4) Applicant submits herewith a copy of the Takahashi et al. reference for the Examiner’s convenience. Taken together, the Holtz-Heppelmann et al. and Takahashi et al. references teach the region – 1032 to nucleotide +33 and therefore provide support for the “region” extending from –1032 to nucleotide +33 as described in the specification and in the current claims. Thus, Applicant submits that the specification does define the metes and bounds of the indicated ‘region.’ Applicant therefore requests withdrawal of this rejection of the claims under 35 U.S.C. §112, first paragraph.

Predictable Correlation With Susceptibility To Autoimmune Disease

Applicant’s previous response of June 1, 2004 emphasized that the instant claims are directed towards susceptibility to autoimmune disease. The Examiner states that “it appears that the response mischaracterizes the previous office action, as the previous office action did not

assert or state that the claims were drawn to a predictable correlation between any disease state or a physiological state.” (August 25, 2004 Office Action, p.9, section 7) Applicant notes that no mischaracterization of the Office Action was intended in the June 1, 2004 response and regrets any unintentional phrasing which could be so interpreted. However, Applicant notes that again in the current office action (August 25, 2004), the Examiner includes language emphasizing disease rather than susceptibility. For example, the section of the office action entitled “Presence and Absence of Working Examples” includes the statement “...such analysis does not predictably establish a correlation between a specific genotype and any autoimmune disease or cancer in general, or even any specific autoimmune disease or cancer.” (p.7) The current office action variously refers to “susceptibility to any autoimmune disease or any cancer” (for example on p.3, 4, 5) and “determining any autoimmune disease or cancer” (p.5), “associated with any autoimmune disease or any cancer” (p.5), and “association with diseases or disease states” (p.6). In light of the inclusion of the such statements emphasizing an association between a polymorphism and disease rather than susceptibility to autoimmune disease, Applicant submits that Applicant was unsure of the Examiner’s position and emphasizes susceptibility in an effort to clarify the issues involved in the current prosecution.

Regarding the substantive issue, the Examiner finds that “in the instant specification, conflicting evidence is provided as to an association between the presence of a T at position – 844 of Fas ligand and susceptibility to rheumatoid arthritis and SLE patients vs. controls.” And that “given such evidence, it is clear that the specification does not support that a generalized predictable correlation can be made between the polymorphisms at position –844 and susceptibility to any autoimmune disease or cancer...” (August 25, 2004 Office Action, p. 10, section 7)

Firstly, Applicant amends claim 1 to remove the reference to cancer. Applicant expressly reserves the right to pursue claims relating to cancer in future.

Next, regarding autoimmune disease, Applicant submits that data presented in the instant specification do in fact support a generalized predictable correlation between the polymorphisms at position –844 and susceptibility to any autoimmune disease. In particular, Applicant states that data presented in Table 1 of the instant specification support a correlation of a polymorphism at –844 with SLE, that data presented in Table 1 concerning rheumatoid arthritis do not conflict with this correlation, and that SLE is a prototypic autoimmune disease such that correlation of the polymorphism at –844 with SLE is generalizable to correlation with susceptibility to other autoimmune disease.

Applicant submits a Declaration made by the inventor, Dr. Robert Kimberly, which indicates that a finding of “conflicting evidence” between results presented pertaining to SLE and those presented pertaining to rheumatoid arthritis would in fact be an erroneous interpretation of the data presented in the present specification. In particular, Dr. Kimberly states that “Table 1 shows a statistically significant difference for SLE vs. control ...” but also that “to conclude a negative result in Table 1 for RA would be a false negative because of a lack of statistical power.” (Declaration of Robert P. Kimberly dated 11/18/04) Thus, although the results obtained pertaining to RA are inconclusive with regard to an association between RA and a polymorphism at -844 in the FasL gene, they do not conflict with the results obtained pertaining to SLE showing such a correlation.

Applicant has previously provided evidence that SLE is a prototypic autoimmune disease such that discovery that a FasL promoter polymorphism is associated with susceptibility to SLE supports an association of that polymorphism with autoimmune disease generally. In response the Examiner asserts that “SLE is actually distinguished from other types of autoimmune diseases.” (August 25, 2004 Office Action, p. 12, section 7) The basis for the Examiner’s distinction appears to be that one of the submitted references (Vyse and Kotzin) teaches that while “SLE is considered to be the prototypic autoimmune disease” the reference also teaches that “unlike specific autoimmune diseases such as MS and type 1 diabetes mellitus, SLE has the potential to involve multiple organ systems directly...” (August 25, 2004 Office Action, p. 12, section 7) The Examiner concludes that “such statement, as well as the references cited by the declaration fail to provide evidence that mutations in FasL which are associated with susceptibility to SLE or diagnostic for SLE, are also associated with susceptibility to autoimmune diseases generally.” (August 25, 2004 Office Action, p. 12, section 7)

In response Applicant provides evidence in the form of the Declaration of the inventor, Dr. Robert Kimberly. The Declaration reiterates that Systemic Lupus Erythematosus (SLE) is a prototypic autoimmune disease of complex multifactorial origin and describes the classification of systemic or organ-specific autoimmune diseases as “generally relevant to clinically observable aspects of autoimmune disease rather than the underlying factors conferring susceptibility to autoimmune disease.” The inventor’s Declaration further describes an underlying molecular basis for autoimmunity involving “production of antibodies and/or immune cells which recognize [an] antigen.” The Declaration further states that a “basis for organ-specific autoimmune disease is generation of antibodies and immune cells which recognize an organ-specific antigen” and that “an aspect of systemic autoimmune disease is

generation of antibodies and immune cells which recognize antigens widespread in the affected organism.” Thus, a conclusion drawn from this evidence is that an aspect of characterization of an autoimmune disease as systemic or organ-specific depends on whether the antigen to which the immune response is directed is a widespread or organ-restricted antigen. This evidence is important in the context of the present invention which involves polymorphisms in the FasL gene since, as the inventor states, the “Fas/FasL system is known to play a role in regulation of immune cells.” In particular, Fas/FasL mediated apoptosis is a mechanism for regulation of immune cells recognizing autoantigens, both widespread or organ-restricted autoantigens. Thus, “where Fas/FasL mediated apoptosis is inhibited, a usual mechanism for reducing the number of autoimmune cells is inhibited, with a resulting susceptibility to autoimmune pathology.” Given this evidence, the distinction between systemic and organ-specific autoimmune disease does not contradict, but again supports the claim that a FasL polymorphism which correlates with susceptibility to SLE is generalizable to other autoimmune disease.

Further evidence that SLE is a prototypic autoimmune disease and that correlation of a susceptibility factor with SLE is generalizable to other autoimmune disease is found in references previously submitted as evidence on this point. For example, in a reference previously submitted as evidence, T. J. Vyse and B.L. Kotzin, Genetic Susceptibility To Systemic Lupus Erythematosus, SLE is described as “the prototypic systemic autoimmune disease.” (Introduction, p.262) However, as detailed above, the Examiner points to the statement in the reference stating that “SLE is actually distinguished from other types of autoimmune diseases” because “unlike organ-specific autoimmune diseases such as multiple sclerosis and type 1 diabetes mellitus, SLE has the potential to involve multiple organ systems directly and its clinical manifestations are extremely diverse and variable.” (Introduction, p.262) However, the Vyse and Kotzin article also teaches that “SLE is a complex genetic trait with contributions from major histocompatibility complex (MHC) genes and multiple non-MHC genes. It is therefore similar to other autoimmune diseases such as type I diabetes mellitus and multiple sclerosis.” (Introduction, p.263) Further, the Vyse and Kotzin article states that “[i]dentification of the genes that predispose to SLE will almost certainly provide important insight into the development of autoimmunity and the cause of autoimmune disease.” (Conclusion, p.285) Thus, the same article that distinguishes systemic and organ-specific autoimmune disease on the basis of clinical observations finds that a systemic autoimmune disease, SLE, is similar to organ-specific disease at the level of molecular etiology.

Applicant submits that in light of the argument and amendment, the claims are fully enabled such that one skilled in the art would be able to make and/or use the invention. Applicant therefore requests withdrawal of the rejection of the claims under 35 U.S.C. §112, first paragraph.

**Remarks Directed to Rejection of Claims 1, 3, and 5 under
35 U.S.C. §112, Second Paragraph**

Claim 1 is rejected as indefinite in reciting a “Fas ligand promoter region extending from nucleotide –1032 to nucleotide +33, wherein a T at position –844.. because the nucleotide positions set forth in the claim are arbitrary absent a sequence for comparison.” (August 25, 2004 Office Action, p.14, section 8)

However, the application discloses that the numbering system used to designate the promoter region and particular nucleotide identities within the promoter region is an art recognized numbering system. The specification states that the “5’ promoter region sequence of the Fas ligand [is] known” and cites C. J. Holtz-Heppelmann et al., J. Biol. Chem. 1998, 273(8):4416-4423. (p. 40, lines 2-5) In addition, the specification includes the information that “[t]he human FasL gene consists of four exons and spans about 8 kb on chromosome 1q23” citing T. Takahashi et al., *International Immunology* 1994, 6(10):1567-74. (p.7, lines 2-4) Applicant submits herewith a copy of the Takahashiet al. reference for the Examiner’s convenience. Taken together, the Holtz-Heppelmann et al. and Takahashi et al. references teach the region –1032 to nucleotide +33 and provide support for the numbering system used to designate the promoter region and particular nucleotide identities within the promoter region in the present application.

Thus, Applicant submits that the specification does define the metes and bounds of the indicated ‘region.’ Applicant therefore requests withdrawal of this rejection under 35 U.S.C. §112, second paragraph.


Claim 5 is rejected under 35 U.S.C. §112, second paragraph as indefinite for the reason that “it is unclear how the claim further limits claim 1 or if the claim is meant to indicate that haplotyping in claim 1 further encompasses haplotyping at positions –756, –478 and –205.” (August 25, 2004 Office Action, p.14, section 8) Applicant has amended the claim for purposes of clarification. Applicant submits that claim 5 is not indefinite and therefore requests withdrawal of this rejection under 35 U.S.C. §112, second paragraph.

Claim 11 was rejected under 35 U.S.C. §112, second paragraph as indefinite because "it is unclear which claim it depends from, as claim 8 is drawn to a non-elected invention." (August 25, 2004 Office Action, p.14, section 8). Applicant hereby cancels claims 7 and 11 and reserves the right to later pursue these and other claims.

Summary

Claims 1, 3, and 5 are the claims currently being examined in this application. Each claim is believed to be in proper form and directed to allowable and patentable subject matter. Applicant submits that the current amendment places the case in better condition for allowance, or in the alternative, in better form for appeal and therefore requests entry of this amendment, reconsideration and allowance of the claims.

Respectfully submitted,

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Human Fas ligand: gene structure, chromosomal location and species specificity

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Abstract

Fas ligand (FasL) is a 40 kDa type II membrane protein belonging to the tumor necrosis factor family, which induces apoptosis by binding to its receptor, Fas. In this report, we isolated the chromosomal gene for human FasL. The human FasL gene consists of ~8.0 kb and is split into four exons. The human FasL gene was mapped on chromosome 1q23 by *in situ* hybridization against human metaphase chromosomes. Human FasL cDNA was isolated by the reverse polymerase chain reaction of mRNA prepared from human activated peripheral blood lymphocytes. Human FasL is a type II membrane protein consisting of 281 amino acids with a calculated M_r of 31,759. It has an identity of 76.9% at the amino acid sequence level with mouse FasL. Both human and mouse recombinant FasL expressed in COS induced apoptosis in the cells expressing either human Fas or mouse Fas, indicating that FasL fully cross-reacts between human and mouse. A comparison of human and mouse FasL chromosomal genes indicated that a ~300 bp sequence upstream of the ATG initiation codon is highly conserved between them. Several transcription *cis*-regulatory elements such as SP-1, NF- κ B and IRF-1 were recognized in this region.

Introduction

Homeostasis in animals is controlled not only by the proliferation and differentiation of cells, but also by cell death (1,2). Cell death during tissue development is programmed and occurs by apoptosis (3). For example, >95% of precursor T cells die by apoptosis during thymic development (4). Blood cells such as activated lymphocytes and macrophages or senescent cells in various tissues are eliminated by apoptosis after they have served their functions (1). In addition to the cell death that occurs during development, other types of cell death are also mediated by apoptosis. For example, in some cases, the cytotoxicity of cytotoxic T lymphocytes (CTL) or natural killer cells is mediated by apoptosis (5). Tumor necrosis factor (TNF) or lymphotoxin (LT) causes apoptosis of target cells (6).

Fas antigen (Fas) is a cell-surface protein belonging to the TNF/nerve growth factor (NGF) receptor family and it mediates apoptosis (7,8). We identified rat and mouse Fas ligand (FasL), and showed that FasL is a member of the TNF family (9-11). Recombinant FasL expressed in COS cells induced apoptosis by binding to Fas, indicating that FasL is a death

factor and that Fas is its receptor (12). Loss-of-function mutations of mouse Fas and FasL were identified as the spontaneous mouse mutations, *lpr* (lymphoproliferation) and *gld* (generalized lymphoproliferative disease) respectively (11,13). Since mice homozygous at the *lpr* or *gld* locus develop lymphadenopathy, and suffer autoimmune disease (14), it is likely that Fas and FasL play an important role in development of T cells.

In addition to lymphocytes, Fas is expressed in various non-lymphoid tissues such as the liver, ovary and lung (8). Fas is also expressed in various carcinoma cells (7,15). On the other hand, FasL mRNA was detected in activated splenocytes (9), and some CTL cell lines such as PC60-d10S express FasL on the cell surface and kill target cells in a Fas-dependent manner (16,17). The CTL in peritoneal exudate lymphocytes or in mixed lymphocyte culture also exert Fas-dependent cytotoxicity (16,18). These results indicate that FasL is involved in CTL-mediated cytotoxicity. Since the administration of agonistic anti-Fas antibody into mice caused hepatic failure, and rapidly killed the animals, we postulated

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that pathological tissue damage such as human fulminant hepatitis may be caused by FasL expressed in activated CTL (12,19).

In this study, we cloned the chromosomal gene and the cDNA for human FasL. The structure of the gene was similar to that of other members of the TNF family such as TNF- α and LT- β . However, unlike these, which are closely arranged on human chromosome 6, the human FasL gene was localized to chromosome 1. The amino acid sequence of human and mouse FasL was well conserved, and no species-specificity was observed between human and mouse FasL.

Methods

Cloning of the human chromosomal gene encoding FasL

A human gene library in λ EMBL3 SP6/T7 constructed with DNA from human placenta was purchased from Clontech Laboratories (Palo Alto, CA). The library was screened by plaque hybridization using the 32 P-labeled 440 bp or the 190 bp DNA fragment carrying the C-terminal (nucleotide 525–967) or N-terminal portion of rat FasL (nucleotide 43–233) respectively (9) as a probe. Hybridization proceeded under low stringency as described (11) and positive clones were plaque-purified. Recombinant λ DNA was prepared as described (20), and subjected to restriction enzyme mapping and Southern hybridization analysis. Appropriate DNA fragments of the recombinant λ DNA were subcloned into pBluescript II (Stratagene, La Jolla, CA), and exons were localized by Southern hybridization using rat FasL cDNA. The DNA sequence was determined using a DNA sequencer (model 370A; Applied Biosystems, Foster City, CA) and the Taq DyeDeoxy cycle sequencing kit from Applied Biosystems. Where appropriate, synthetic oligonucleotides were used as specific primers.

Cloning of human FasL cDNA by polymerase chain reaction (PCR)

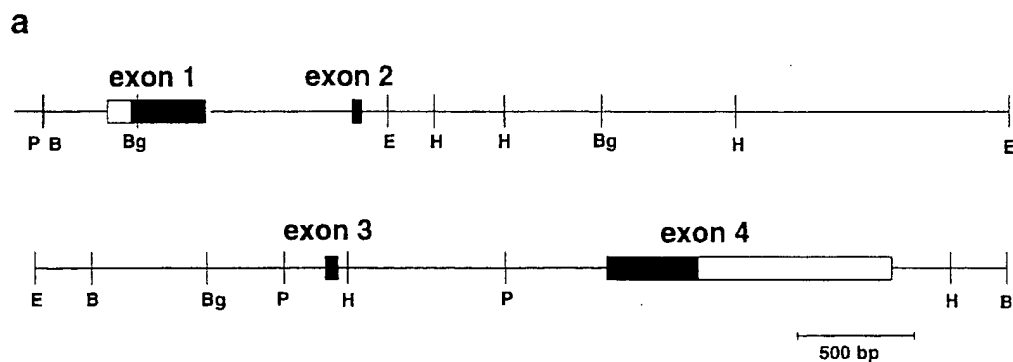
Human peripheral blood was obtained from a healthy adult volunteer (T. S.). The peripheral blood lymphocytes (PBL) were obtained by centrifugation on Nycodenz solution (NycodenzTM 1.077; Nycomed, Oslo, Norway), and cultured in AIM-V medium (Pharmacia, Uppsala, Sweden) containing 5 μ g/ml concanavalin A (ConA) and 20 ng/ml of human IL-2 (provided by Ajinomoto Co., Tokyo, Japan) for 24 h. The cells

were then grown for 6 days in AIM-V medium containing 20 ng/ml of human IL-2, and finally stimulated with 10 ng/ml phorbol myristate acetate and 500 ng/ml ionomycin for 8 h.

Poly(A) RNA was prepared from activated PBL using a mRNA isolation kit from Pharmacia and single-stranded cDNA was synthesized with random hexamer oligonucleotides as primers. In brief, 1.0 μ g of poly(A) RNA was incubated at 42°C for 60 min with RNaseH⁻ M-MLV reverse transcriptase (Superscript II; GibcoBRL, Gaithersburg, MD) with 50 ng of d(N)₆ as a primer in a total volume of 20 μ l. After incubation, a 2 μ l aliquot of the reaction mixture was diluted with 100 μ l of PCR buffer (21), and human FasL cDNA was amplified by PCR using a sense primer carrying a 20 nucleotide sequence (CTACAGGACTGAGAAGAAGT) upstream of the ATG initiation codon and an antisense primer carrying a 20 nucleotide sequence (ACATTCTCGGTGCCTGTAAC) downstream of the TAA termination codon. An *Xba*I recognition sequence (GCTCTAGA) was added to the 5' end of each primer. The reaction mixture for PCR contained 100 pmol each of the sense and antisense primers, and the reaction was started by adding 2.5 units of *Thermus aquaticus* DNA polymerase (Taq polymerase). The conditions for PCR were 1.0 min at 94°C, 2.0 min at 55°C and 3 min at 72°C for 20 cycles. The PCR product was digested with *Xba*I and ligated into pBluescript II.

Fluorescent in situ hybridization (FISH)

FISH was performed essentially as described (22). In brief, human (pro)metaphase chromosomes were prepared from normal male lymphocytes using the thymidine synchronization, BrdU release technique for the delineation of G-bands. Before hybridization *in situ*, chromosomes were stained in Hoechst 33258 and irradiated with UV. Recombinant λ DNAs (λ HFL4 and 5) carrying human FasL chromosomal region were labeled with biotin-16-dUTP by nick-translation and hybridized to the denatured chromosome on slides at a final concentration of 25 ng/ μ l in a mixture of 50% formamide, 10% dextran sulfate (Sigma, St Louis, MO), 2 \times SSC, Cot-1 DNA (GibcoBRL; 0.2 μ g/ μ l), sonicated salmon sperm DNA (2 μ g/ μ l) and *Escherichia coli* tRNA (2 μ g/ μ l). The hybridization signals were detected with FITC-avidin (Boehringer, Mannheim, Germany) and chromosomes were counterstained with 1 μ g/ μ l propidium iodide. The precise signal position was determined by the delineation of G-banding patterns (22).



b

AATTATAATGTATAAAAAAGCATGCAATTATAATTCATAAAATATAGCCCCACTGACCATTCCTCTGTAGCTGGGAGCAGTTTCACTAACAGGGCTA
 TACCCCATGCTGACCTGCTCTGCAGGATCCCAGGAAGGTGAGCATAGCTTACTAAGCTGTTTGGGTAGCACAGCGACAGCAACTGAGGCCCTTGAAGGC
 TGTATCAGAAAATTGTGGGCGGAACTTCCAGGGGTTTGTCTGAGCTTCTTGAGGCTTCTCAGCTTCAGCTGCAAAGTGAGTGGGTGTTCTTTGAG
 AAGCAGAATCAGAGAGAGAGAGATAGAGAAAGAGAAAGACAGAGGTGTTTCCCTTAGCTATGGAACTCTATAAGAGAGATCCAGCTTGCCTCCTCTTG
 AGCAGTCAGCAACAGGGTCCCGTCTCTGACACCTCAGCCTCTACAGGACTGAGAAGAAGTAAACCGTTTGCTGGGGCTGGCCTGACTCACCAGCTGCC

20

Met Gln Gln Pro Phe Asn Tyr Pro Tyr Pro Gln Ile Tyr Trp Val Asp Ser Ser Ala Ser Ser Pro Trp Ala Pro
 ATG CAG CAG CCC TTC AAT TAC CCA TAT CCC CAG ATC TAC TGG GTG CAC AGC AGT GCC AGC TCT CCC TGG GCC CCT

40

Pro Gly Thr Val Leu Pro Cys Pro Thr Ser Val Pro Arg Arg Pro Gly Gln Arg Arg Pro Pro Pro Pro Pro Pro
 CCA GGC ACA GTT CTT CCC TGT CCA ACC TCT GTG CCC AGA AGG CCT GGT CAA AGG AGG CCA CCA CCA CCA CCG CCA

60

Pro Pro Pro Leu Pro Pro Pro Pro Pro Pro Leu Pro Pro Leu Pro Leu Pro Pro Leu Lys Lys Arg Gly
 CCG CCA CCA CTA CCA CCT CCG CCG CCG CCG CCA CCA CTG CCT CCA CTA CCG CTG CCA CCC CTG AAG AAG AGA GGG

80

Asn His Ser Thr Gly Leu Cys Leu Leu Val Met Phe Phe Met Val Leu Val Ala Leu Val Gly Leu Gly Leu Gly
 AAC CAC AGC ACA GGC CTG TGT CTC CTT GTG ATG TTT TTC ATG GTT CTG GTT GCC TTG GTA GGA TTG GGC CTG GGG

100

Met Phe Gln Leu Phe His Leu Gln Lys Glu Leu Ala Glu Leu Arg Glu
 ATG TTT CAG CTC TTC CAC CTA CAG AAG GAG CTG GCA GAA CTC CGA GAG GTAAGCCTGCCGGCAGACTGCTGTG...intron.1

120

Ser Thr Ser Gln Met His Thr Ala Ser Ser Leu Glu Lys Gln Ile G
 ..ATCTTTTCTCTTCTGTTTACTAG TCT ACC AGC CAG ATG CAC ACA GCA TCA TCT TTG GAG AAG CAA ATA G GTGAGTCTT

140

ly His Pro Ser Pro Pro Pro Glu Lys Lys Glu
 TTTTCGATGTACAT...intron 2....TATTTTCTCTCTCTATGATACAG GC CAC CCC AGT CCA CCC CCT GAA AAA AAG GAG

Leu Arg Lys Val Ala His Leu Thr G
 CTG AGG AAA GTG GCC CAT TTA ACA G GTCTGTATCTGGAAGGTACAGGTGA....intron 3....AAAGCTCCTTTGGATTATTTCAG

160

ly Lys Ser Asn Ser Arg Ser Met Pro Leu Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val Lys
 GC AAG TCC AAC TCA AGG TCC ATG CCT CTG GAA TGG GAA GAC ACC TAT GGA ATT GTC CTG CTT TCT GGA GTG AAG

180

Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln
 TAT AAG AAG GGT GGC CTT GTG ATC AAT GAA ACT GGG CTG TAC TTT GTA TAT TCC AAA GTA TAC TTC CCG GGT CAA

200

Ser Cys Asn Asn Leu Pro Leu Ser His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu Val Met Met
 TCT TGC AAC AAC CTG CCC CTG AGC CAC AAG GTC TAC ATG AGG AAC TCT AAG TAT CCC CAG GAT CTG GTG ATG ATG

220

Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met Trp Ala Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn
 GAG GGG AAG ATG ATG AGC TAC TGC ACT ACT GGG CAG ATG TGG GCC CGC AGC AGC TAC CTG GGG GCA GTG TTC AAT

240

Leu Thr Ser Ala Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu Glu Ser Gln Thr Phe
 CTT ACC AGT GCT GAT CAT TTA TAT GTC AAC GTA TCT GAG CTC TCT CTG GTC AAT TTT GAG GAA TCT CAG ACG TTT

260

Phe Gly Leu Tyr Lys Leu
 TTC GGC TTA TAT AAG CTC TAA GAGAAGCACTTTGGGATTCTTTCCATTATGATTCTTTGTTTACAGGCACCGAGAATGTTCTATTTCAGTGAGG

GTCTTCTTACATGCATTGTGAGGTCAAGTAAGAAGACATGAACCAAGTGGACCTTGAGACCACAGGGTTCAAATGTCTGTAGCTCCTCAACTCACCTAA
 TGTTTATGAGCCAGACAAATGGAGGAATATGACCGAAGAACATAGAACTCTGGGCTGCCATGTGAAGAGCCGAGAAGCATGAAAAAGCAGCTACCAGGTG
 TTCTACACTCATCTTAGTGCCTGAGAGTATTTAGGCAGATTGAAAAGGACACC

Fig. 1. The gene structure of human FasL. (a) The gene organization of human FasL. The organization of the human FasL gene is shown schematically. Boxes and the lines between them represent four exons and three introns respectively. The filled areas represent the coding sequence, while the non-coding regions are indicated by open areas. The recognition sites for *Pst*I (P), *Bam*HI (B), *Bgl*II (Bg), *Eco*RI (E) and *Hind*III (H) are shown. (b) The genomic sequence of human FasL. The nucleotide sequence of exons and 5' promoter region of the human FasL is shown with the predicted amino acid sequence for the exons. Amino acids are numbered starting at Met-1. The TATA box is underlined and the primers for PCR are indicated by arrows. These sequence data are available from EMBL/GenBank/DBJ under accession number 411821.

Assay of cytotoxic activity

The *Xba*I DNA fragment carrying human or mouse FasL cDNA was inserted into the mammalian expression vector pEF-BOS (23). Monkey COS cells cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS were transfected with the expression plasmid using the DEAE-dextran method (24). Forty eight hours later, the cytotoxic activity of the transfected COS cells was determined as described previously (9). In brief, mouse WR19L and its transformants expressing mouse Fas (W4) (19) or human Fas (WC8A) (25) were labeled with ^{51}Cr . The ^{51}Cr -labeled cells (1×10^4) were incubated for 4 h at 37°C with the transfected COS cells at various ratios in round-bottomed microtiter plates in a total volume of 200 μl . After incubation, the plates were centrifuged and the radioactivity in 100 μl aliquots of the supernatants was determined. The spontaneous release of ^{51}Cr was determined by incubating the target cells with medium alone, whereas the maximum release was determined by adding Triton X-100 to a final concentration of 0.1%. The percent specific lysis was calculated as follows: [(experimental ^{51}Cr release - spontaneous ^{51}Cr release) / (maximum ^{51}Cr release - spontaneous ^{51}Cr release) $\times 100$]. The spontaneous release of ^{51}Cr was routinely 8–10% of the maximum release.

The soluble forms of mouse Fas (mFas-Fc) or human Fas (hFas-Fc) were prepared by expressing the hybrid gene consisting of the extracellular region of mouse or human Fas fused to the Fc region of the human Ig heavy chain (IgH) as described previously (10). mFas-Fc and hFas-Fc were purified using Protein A-Sepharose and added to the assay mixture to examine their inhibitory effects.

Results*Chromosomal gene for human FasL*

A human genomic library constructed with human placenta DNA and $\lambda\text{EMBL-3 SP6/T7}$ vector was screened under low stringency with the 5' or 3' part of rat FasL cDNA as a probe, which resulted in three positive clones (λHFL4 , 5 and 7). Since λHFL5 gave positive signals with both 5' and 3' probe DNAs, it was further characterized by restriction enzyme mapping and Southern hybridization. The nucleotide sequence of the human FasL genomic region determined after subcloning into pBluescript II is presented in Fig. 1. A comparison of the human genomic DNA sequence with the rat FasL cDNA (9) and human FasL cDNA obtained by reverse PCR (see below) revealed the structural organization of the human FasL gene (Fig. 1). It consists of ~8.0 kb and is split into four exons. All of the splice donor and acceptor sites conformed to the GT-AG rule, and further flanking sequences were in good agreement with favored nucleotide frequencies noticed in other split genes (26). The gene organization of human FasL including the position of introns was the same as that of mouse FasL (11), and similar to that of other members of the TNF family which include TNF- α and LT- β (27,28).

Assignment of human FasL gene to 1q23

Assignment of several (pro)metaphase chromosome spreads localized FasL λDNAs (λHFL4 and 5) to human chromosome 1q23. To precisely sublocalize FasL on the region 1q23, 10

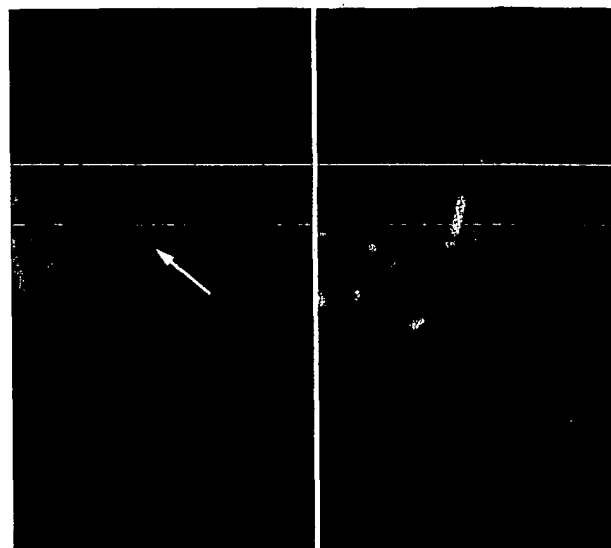


Fig. 2. FISH of the human FasL gene. (Left) Partial metaphases showing the FITC signals (arrow) on both chromatids of chromosome 1. (Right) The G-banding pattern of the same chromosome, indicating that the FITC signal is at 1q23.3.

prometaphase chromosomes 1 exhibiting twin-spots of FITC signals were visualized through a B-2A filter (Nikon, Tokyo, Japan) and then G-band patterns on the same chromosomes were delineated through a UV-2A filter (Nikon, Tokyo, Japan). As shown in Fig. 2, the signals were sublocalized at 1q23.3 of the long arm of human chromosome 1.

Primary sequence of the human FasL

Activated rat or mouse splenocytes express FasL mRNA (9,11). To obtain human FasL cDNA, poly(A) RNA was prepared from human PBL activated with Con A and IL-2. The human FasL cDNA was then amplified by reverse PCR using appropriate primers representative of the 5' or 3' non-coding regions of the human FasL gene (Fig. 1). The resultant 970 bp PCR product was inserted into pBluescript II and its nucleotide sequence was determined. The sequence was completely identical to that of exons of the human FasL chromosomal gene and contained a long open reading frame of 843 bp. The reading frame codes for a polypeptide of 281 amino acids with a calculated M_r of 31,759. As in rat and mouse FasLs (9,11), human FasL is also a type II membrane protein, and it is highly homologous to mouse and rat FasLs. Alignment of their amino acid sequences showed that human FasL has an identity of 76.9 and 75.8% with mouse FasL and rat FasL respectively (Fig. 3). When conservative amino acid replacement is considered as homologous, the similarity of human FasL with mouse FasL and rat FasL increased to 86.2 and 84.0% respectively.

No species specificity of FasL between human and mouse

The human FasL cDNA was inserted into a mammalian expression vector and introduced into COS cells. As shown in Fig. 4, the COS cells transfected with human FasL cDNA

	20	40	60
hFasL:	MQQPFNYYPQIYWVDSSASSPW	APPGT	VLPCTSPVRRPGQRRPPPPPPPLPPPPPP
mFasL:	MQQPMNYPCPQIFWVDSSATSSW	APPGSVFPCPCGPRGPDQRRPPPPPPVS-	PLPPPS
rFasL:	MOOPVNYPCPQIYWVDSSATSPW	APPGSVFSCPSSGPRGPGQRRPPPPPPPS-	PLPPPS
	80	100	120
hFasL:	PPLPPLPPLPPLKRGNHSIGLCLLVMPFIVLVALVGLGLGMP	QLFHLQKELAE	RESTSQ
mFasL:	QPLPPLPPLTPLKKKDN-TNLNLPLVPVFPFIVLVALVGLGLGMP	QLFHLQKELAE	REFTNQ
rFasL:	QPPPLPPLSPLKKKDN--TELNLPVITFPFIVLVALVGLGLGMP	QLFHLQKELAE	REFTNH
	140	160	180
hFasL:	MHTASSLEKQIGHPSPPPEKKELRKVAHLT	TGKSNSRSMPL	EWEDTYGIVLLSGVKYKKGG
mFasL:	SLKVSSFEEKIANPSTPSEKKEPRSAHLT	TGNPHSRSIPL	EWEDTYGTALISGVKYKKGG
rFasL:	SLRVSSFEKQIANPSTPSETKKPRSAHLT	TGNPRSRSIPL	EWEDTYGTALISGVKYKKGG
	200	220	240
hFasL:	LVINETGLYFVYSKVYFRGQSCNNLPLSHKVYMRNSKYPQDLVMEGKMMSYCTTGQMW		
mFasL:	LVINETGLYFVYSKVYFRGQSCNNQPLNHKVYMRNSKYPEDLVMEEKRLNYCTTGQIWA		
rFasL:	LVINEAGLYFVYSKVYFRGQSCNSQPLSHKVYMRNFKYPGDLVMEKKLNCTTGQIWA		
	260	280	
hFasL:	RSSYLGAVFNLTSADHLYVNVSELSLVNFEE	SQTFFGLYKL	
mFasL:	HSSYLGAVFNLTSADHLYVNISQLSLINFEE	SKTFFGLYKL	
rFasL:	HSSYLGAVFNLTVADHLYVNISQLSLINFEE	SKTFFGLYKL	

Fig. 3. Comparison of the amino acid sequences of human FasL, mouse FasL and rat FasL. The amino acid sequences of human FasL (hFasL), mouse FasL (mFasL) (11) and rat FasL (rFasL) (9) are aligned to give maximal homology by introducing several gaps (-). Numbers indicate the amino acid number for human FasL. The amino acid residues conserved in three species are underlined. The proline residues in the cytoplasmic region of the FasL are shown in bold letters, while the amino acids in the putative transmembrane domain are presented in shadowed letters.

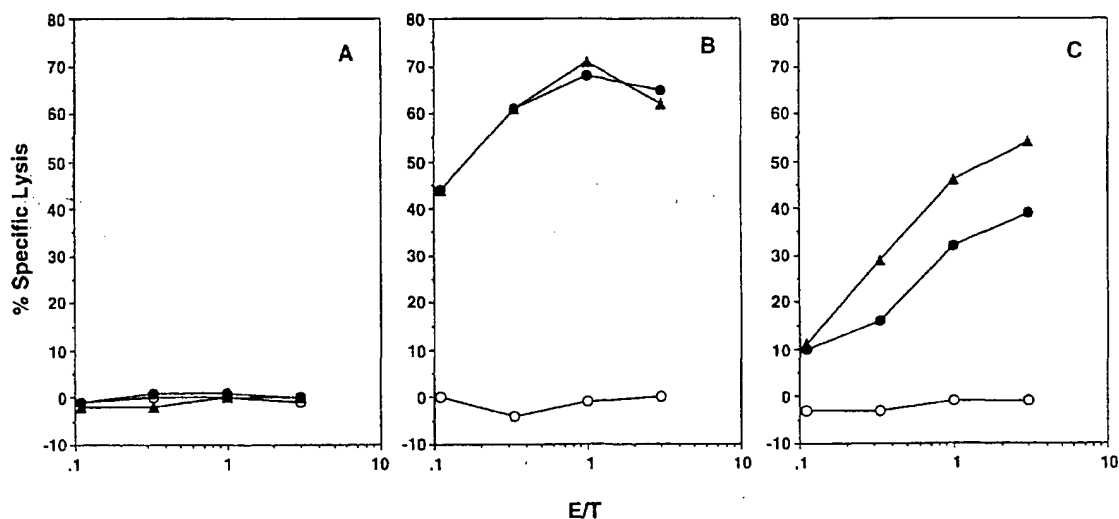


Fig. 4. Cytotoxic activities of human and mouse FasL. COS cells were transfected with pEF-BOS vector (○), or pEF-BOS carrying human FasL (●) or mouse FasL cDNA (▲). After 48 h, the cytotoxicity of the transfected COS cells was determined using WR19L cells (A), W4 cells (B) or WC8A cells (C) as target cells as described under Methods. The assays were done in duplicate and the values agreed within 10% error. The average values are plotted.

lysed the transformants expressing mouse Fas (W4) or human Fas (WC8A) in a dose-dependent manner. The recombinant human FasL expressed in COS cells did not show any cytolytic activity on parental WR19L cells which hardly express Fas. Similarly, mouse FasL expressed in COS cells lysed both W4

and WC8A cells as efficiently as human FasL. These results indicated that human FasL can bind to mouse as well as human Fas and *vice versa*. To confirm the lack of species specificity of the Fas system between human and mouse, the soluble forms of human Fas (hFas-Fc) and mouse Fas (mFas-

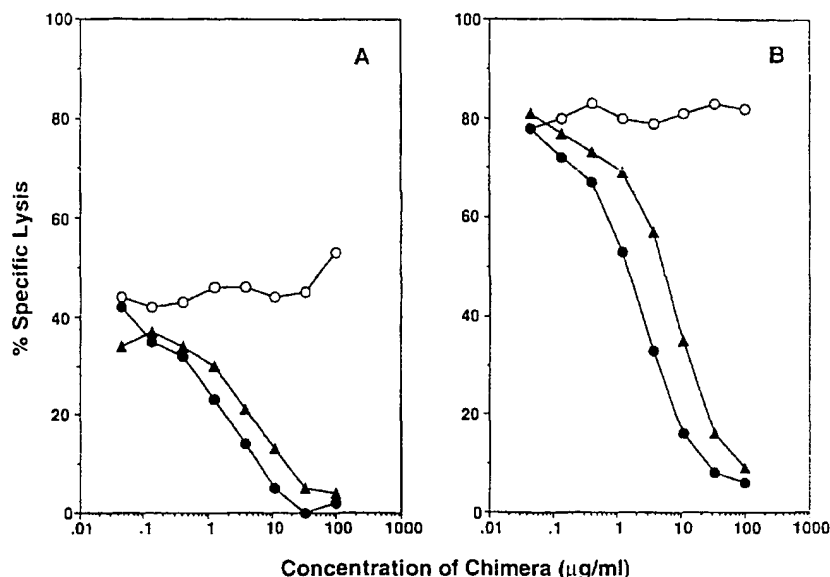


Fig. 5. Inhibition of the human FasL-induced cytotoxicity by the soluble form of Fas. COS cells were transfected with the human FasL expression plasmid. The cytotoxic activity of the recombinant FasL was then assayed using WC8A cells (A) or W4 cells (B) as target cells at the E/T (effector cells/target cells) ratio of 0.75. The indicated concentrations of the soluble form of human type I TNF receptor (hTNFR β -Fc, ○), human Fas (hFas-Fc, ●) or mouse Fas (mFas-Fc, ▲) were added to the assay mixture. The assays for cytotoxicity were done in duplicate and the average values are plotted. The difference in duplicate was within 20%.

Fc) were prepared as described previously (10). As shown in Fig. 5, both hFas-Fc and mFas-Fc inhibited the human FasL-induced cytotoxicity of WC8A or W4 cells in a similar dose-dependent manner. On the other hand, the soluble form of human TNF receptor type I (hTNFR β -Fc) did not inhibit the human FasL-induced cytotoxicity.

Discussion

The mouse FasL gene is localized on chromosome 1 and the mouse *gld* mutant carries a mutation in the FasL (11). Here, human FasL gene was mapped on chromosome 1q23.3 which is homologous to the *gld* locus of the mouse, confirming our previous assignment of FasL to the *gld* mutation. Mice homozygous in the *gld* mutation show lymphadenopathy and autoimmune disease. It is possible that similar human diseases such as angioimmunoblastic lymphadenopathy (29) are caused by a loss-of-function mutation in FasL or Fas. Furthermore, the fact that the FasL and Fas mediate apoptosis suggests that these genes function as tumor suppressor genes. T cell lymphoma or other malignant cells should be investigated to determine whether they carry an abnormality in the FasL or Fas gene. The FasL is a member of the TNF family. Accordingly, the chromosomal gene structure of FasL is similar to those of other members of the TNF family such as TNF- α and LT- β (27,28). The TNF- α and LT- β genes are tandemly arranged near the HLA gene cluster on human chromosome 6p21 (28), whereas the human FasL gene is on chromosome 1q23. Near the FasL gene, there is a gene cluster for CD1 (thymocyte antigen) related to HLA class I (30). These results indicate that a large region of chromosome

including the HLA gene cluster and the ancestral gene for TNF/FasL is duplicated, and that one of them has translocated to another chromosome.

Members of the TNF family have no species specificity between human and mouse (31,32), except that human TNF- α cannot bind mouse type II TNF receptor (33). Here, we showed that the FasL also has no species-specificity between human and mouse. Both human and mouse FasLs induced apoptosis in cells expressing either mouse or human Fas with the same efficiency. Although the overall amino acid sequence of FasL is highly conserved (76.9% identity) between human and mouse (Fig. 3), the similarity between human and mouse Fas is much less pronounced (49.3% identity). More conservation of the ligand than the receptor sequence is also observed in other members of the TNF family.

Among members of the TNF family, the FasL has a rather long N-terminal intracellular region (9,11). We remarked that in rat FasL, this region is very rich in proline residues (9). This property can be seen in human and mouse FasL, but not in other members of the TNF family. The proline-rich sequence has been found in various proteins and binds to the SH3 (*src* homology region 3) domain (34). The consensus sequence for the SH3 binding site was proposed to be XPXPPPP Ψ XP (Ψ represents a hydrophobic amino acid) (34). This sequence can be found in human, mouse and rat FasL. The SH3 domains play important roles in mediating specific protein-protein interactions, specifically in the cytoskeleton (35). Most cytokines are soluble proteins and the binding of cytokines to their receptor induces a specific signal in the target cells. Afterwards, the cytokine-receptor complex is internalized in the target cells and cytokines are degraded in the cells. In

```

hFasL:      ATTCTCCTGTAGCTG-GGAGCAGTTCAC
mFasL:      ACACCCCAG-AGCTGCGGAAGAGCTAAT

                -400
hFasL: ACTAAC-AGGGCTATACCCCATGCTGACCTGCTCTGCAGGATCCCAGGAAGGTGAGCAT
mFasL: GTCCTCAAGGGGTAT---CCAGCGCTGACTTGCTGAGTTGGACCTCAGGCAGGCAAGCCT
                -350

                -300
hFasL: AGCCTACTAACCTGTTTGGGTAGCACAGCGACAGCAACTGAGGCCTTGAAGGCT-GTTAT
mFasL: GGTTTACCAGCCTTCTCAGTTAGCACAGAGACGCCAATTGGAACCTCGAAGACTTGTCGT
                -300

                -250
hFasL: CAGAAAATTGTGGGCGGAAACTTCCAGGGGTTTGTCTGAGCTTCTT-GAGGCTTCTCAG
mFasL: CAGAAATTTCTGGGCGGAAACTTCCCTGGGGTT-GCTGTGAGCTTTTGGAGGCTTCTCAG
                -250      SP-1      NF-κB

                -200
hFasL: CTTCAGCTGCAAAGTGAGTGGGTGTTTCTTTGAGAAGCAGAAATCAGAGA-GAGAG-AGAT
mFasL: CTTCAGATGC-AAGTGAGTGGGTGTTCTCACAGAGAAGCAAA---GAGAAGAGAACAG--
                IRF-1                                -150

hFasL: AGAGAAAGAGAAAGACAGAGGTGTTTCCCTTAGCTATGGAAACTCTATAAGAGAGATCCA
mFasL: -GAGAA-----AGGTGTTTCCCTTAGCTGCGGAAACTTTATAAAGAAAACCTTA
                TATA                                -100

                -100
hFasL: GCTTGCCCTCCTCTTGAGCAGTCAGCAACAGGGTCCCGTCCTTGACACCTCAGCCTCT---
mFasL: GCTT-----CTCTGGAGCAGTCAGCGTCAGAGTTCTGTCTTGACACCTGAGTCTCCTCC
                -50

                -50
hFasL: ACAGGACTGAGAAGAAGTAAACCGTTTGCTGGGGCTGGCCTGACTCACCAGCTGCCATG
mFasL: ACAAGGCTGTGA-GAAG-GAAACCCCTTCTGGGGCTGGG-----TGCCATG

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Fig. 6. Comparison of the 5' flanking regions of human and mouse FasL genes. In aligning the sequences, gaps (-) were introduced to obtain maximal homology. On the nucleotide sequence, number 1 indicates the ATG initiation codon. The nucleotide residues conserved between human and mouse FasL genes are shown by bold letters. The binding sites for SP-1, NF-κB and IRF-1, and the TATA box are underlined.

contrast, the FasL is a type II membrane protein and expressed in activated T cells. The interaction of FasL on the effector cells with Fas on the target cells induces an apoptotic signal in the target cells. What happens to the FasL on the effector cells remains unknown. Although it is possible that the FasL is cleaved from the effector cells and internalized into the target cells, there are other possibilities. The FasL may be down-regulated in the effector cells or it may be phagocytosed by the target cells as found in photoreceptor development in *Drosophila* (36). The proline-rich sequence in the cytoplasmic region of the FasL may play a role in its down-regulation. In any case, it will be of interest to examine whether the SH3 domain can bind this proline-rich region of FasL or not.

The FasL is expressed in activated T lymphocytes. As shown in Fig. 6, the promoter region of human and mouse FasL genes is significantly conserved up to 200 bp from the TATA box. In this region, we can find several *cis*-regulatory elements for transcription factors. At 90 (for human) or 70 bp (for mouse) upstream of the TATA box, there is a binding site for IRF-1. The element for IRF-1, originally identified in IFN genes as elements required for induction of IFN by virus, can be found in the promoter of various IFN-responsive genes (37). Furthermore, since activation of T cells greatly induces the expression of IRF-1 (37) and IRF-1-deficient mice show some defect in T cell development (38), a role for IRF-1 in T-cells has been suggested. It would be of interest to examine whether IRF-1 or its related factors such as IRF-2 or IRF-3 regulate the FasL gene expression in T cells. The promoter

of the FasL gene also contains SP-1 and NF-κB binding sites. SP-1 is a rather ubiquitous factor and it is found in the promoter of many house-keeping genes. On the other hand, many inducible genes for lymphokines and monokines including the TNF gene carry NF-κB or its related NFAT element (39,40), which is indispensable for the induction of the gene. It is likely that the NF-κB element in the FasL gene also plays an important role in the induction of this gene.

In conclusion, we presented the amino acid sequence of human FasL, which is a death factor expressed in CTL. FasL may be involved in various human diseases mediated by autoreactive cytotoxic T cells. The human FasL cDNA would be an important tool with which to elucidate the pathological role of FasL in human disease. FasL seems to be transiently expressed in activated T cells. Using the FasL promoter sequence isolated here, the kinds of signals activate the FasL gene can be examined.

Acknowledgements

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Abbreviations

Con A	concanavalin A
CTL	cytotoxic T lymphocytes
FasL	Fas ligand
FISH	fluorescent <i>in situ</i> hybridization

<i>gld</i>	generalized lymphoproliferative disease
<i>lpr</i>	lymphoproliferation
LT	lymphotoxin
NGF	nerve growth factor
PBL	peripheral blood lymphocytes
PCR	polymerase chain reaction
TNF	tumor necrosis factor

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Robert P. Kimberly

Serial No.: 09/807,501

Group Art Unit: 1634

Filing Date: May 23, 2001

Examiner: Jehanne Souaya Sitton

For: POLYMORPHISM IN FAS PROMOTER AND FAS LIGAND PROMOTER

DECLARATION OF ROBERT P. KIMBERLY
UNDER 37 CFR 1.132Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Robert P. Kimberly, declare as follows:

1. I am the inventor of the invention disclosed in the above-identified application for patent.
2. I state that Table 1 shows a statistically significant difference for SLE vs. control ($p < 0.05$, Chi Square). While data included in Table 1 in the application for patent do not indicate a clear association with rheumatoid arthritis (RA), the sample size is smaller than that for SLE and is, therefore, underpowered -- that is, the numbers are small and with small numbers it is easy to make what statisticians call a Type II error ("false negative"). If you take the numbers for RA and control in Table 1 and simply double just the RA, you get statistically significant results ($p < 0.05$, Chi Square = 4.201) and if you double both the RA and controls you become even more significant ($p < 0.025$, Chi square = 6.018). In other words, to conclude a "negative" result in Table 1 for RA would be a "false negative" because of lack of statistical power (ie, the test sample was very small). Statisticians typically project analytical results by increasing sample

sizes. For example, when done by combining samples, the technique is termed "meta-analysis" and is a standard practice in statistical analysis.

3. I state that Systemic Lupus Erythematosus (SLE) is a prototypic autoimmune disease of complex multifactorial origin. While SLE is often cited as a prototypic systemic autoimmune disease, this classification of autoimmune disease is generally relevant to clinically observable aspects of autoimmune disease rather than the underlying factors conferring susceptibility to autoimmune disease. I submit the following brief summary in order to provide information in the context of the present invention. The process of generating immunity includes stimulation of an immune response in an organism by an antigen. One aspect of an immune response is production of antibodies and/or immune cells which recognize the antigen. A basis for organ-specific autoimmune disease is generation of antibodies and immune cells which recognize an organ-specific antigen. For example, autoimmune thyroiditis is typically considered an organ-specific disease characterized by destruction of thyroid tissue and resulting in hypothyroidism. Autoantibodies in this disease include those directed against thyroglobulin and thyroid peroxidase, proteins expressed preferentially by thyroid cells. In contrast, an aspect of systemic autoimmune disease is generation of antibodies and immune cells which recognize antigens widespread in the affected organism. For example, a typical characteristic of SLE is presence of antibodies directed towards nuclear proteins present in many nuclei throughout the organism. Thus, a basis for characterizing an autoimmune disease as systemic or organ-specific is presence of antibodies and immune cells directed to a widespread or organ-restricted antigen.

The present invention involves polymorphisms in the FasL gene. The Fas/FasL system is known to play a role in regulation of immune cells. In particular, immune cells recognizing autoantigens, both widespread or organ-restricted, are regulated by Fas/FasL mediated apoptosis.

Where Fas/FasL mediated apoptosis is inhibited, a usual mechanism for reducing the number of autoimmune cells is inhibited, with a resulting susceptibility to autoimmune pathology.

4. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date

11/18/04


Robert P. Kimberly

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